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ORIGINAL ARTICLE

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Mast cell tryptase in postmortem serum — reference values and confounders

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Abstract We have investigated the effects of some factors suspected of inducing spuriously increased tryptase concentrations, specifically sampling site, conjunctival petechial bleeding and prone position at the time of death as indicators of premortem asphyxia, and resuscitation efforts by external cardiac massage. Tryptase was measured in blood from the femoral vein in 60 deaths: 39 control cases who died rapidly (within minutes) from natural causes (sudden cardiac death and acute aortic dissection), 16 with death caused by prolonged asphyxia (traumatic compression of the chest and suffocation due to body position or smothering), and five anaphylactic deaths. In 44 of these cases, tryptase was measured in both heart and femoral blood. Mast cell tryptase was analyzed with a commercial FEIA method (Pharmacia Diagnostics AB, Uppsala, Sweden) measuring both α- and β-tryptase. Assuming that tryptase values in the control group were gamma distributed, we calculated the upper normal limits for tryptase concentrations in femoral blood. It was found that 95% of the controls had values below 44.3 μg/l (femoral blood), SD 5.27 μg/l. All but one of the anaphylactic deaths had tryptase concentrations exceeding that limit. Tryptase was significantly elevated in femoral blood from anaphylactic deaths ($p<0.007$), compared with the controls. Also, in the cases where death had occurred due to asphyxia tryptase was elevated in femoral blood ($p<0.04$). A significant difference in tryptase concentrations was seen between blood from the heart and the femoral vessels ($p<0.02$) in the whole material ($n=44$). Tryptase concentrations in femoral blood were not influenced by prone position at death, or resuscitation efforts. It is concluded that asphyxia premortem seems to affect tryptase concentrations, that postmortem tryptase measurements should be done in serum from femoral blood, and that the normal upper limit, covering 95%, is 44.3 μg/l.

Keywords Mast cell · Tryptase · Postmortem · Asphyxia · Anaphylaxis · Allergy

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Introduction

Postmortem measurement of mast cell tryptase in serum is so far the only possible means of diagnosing or confirming death due to anaphylactic shock. Autopsy findings after acute anaphylactic death are nonspecific: pulmonary congestion and edema, visceral congestion, and sometimes, laryngeal edema [1].

Mast cells are activated and degranulated when the receptors on the cell surface are crosslinked by allergen-bound IgE. This induces the release of preformed proteases and histamine into blood and tissues resulting in the typical symptoms of allergy and anaphylaxis [2]. Among the proteases, tryptase has been used as a marker of allergic reactions, as it is more stable and has a longer half-life than histamine [3]. There are mainly two varieties of tryptase, an inactive tetramere (α) and an active free form (β) [4].

Tryptase can also be used to diagnose anaphylaxis postmortem [5], but reference values have been difficult to establish [6], and artifactual elevations that are not well understood, are sometimes seen [7]. Previously investigated factors that may have some influence on postmortem tryptase concentration are (1) postmortem interval (PMI)

and sampling of blood for analysis [6, 8], and (2) hemolysis and trauma [9]. The influence of the PMI is not clear. It had no effect on the tryptase concentrations in one study [6] but correlated with increased tryptase levels in another [8]. Hemolysis, per se, did not influence tryptase neither in postmortem blood samples nor in experimentally induced hemolysis. However, in trauma victims, increased hemolysis showed a positive correlation with increase in tryptase concentrations [9]. Elevated values are often seen in sudden infant death syndrome (SIDS) [10–12], but whether this is due to agonal asphyxia or if it is a postmortem artifact, due to sampling of blood from the heart is not clear [12].

As artifactual values might occur, it is important to investigate possible confounding factors and to establish an upper cut-off value for tryptase measured postmortem.

In the present study, we have attempted to establish reference values for tryptase (α and β) and investigated additional factors believed to influence the tryptase concentration, such as sampling site (i.e., heart blood vs femoral blood), prolonged agonal asphyxia, and resuscitation efforts by means of external cardiac massage.

Materials and methods

Blood was sampled from the femoral veins (n=60) and from the ventricles of the heart (n=44) in a total of 60 autopsy cases—12 females and 48 males, mean age 44±17 standard deviation (SD), range 8–84 years. The time between death and autopsy varied from 3 to 5 days.

The protocol included data on the position in which the deceased was found, any presence of petechial hemorrhages in the conjunctivae and/or face, and whether resuscitation by means of external cardiac massage had been attempted (Table 1).

During the study period, five anaphylactic deaths were registered. The main criterion for anaphylactic death was that it followed immediately or very soon after contact with an identified allergen or therapeutic agent. Secondary criteria were increased levels of tryptase and identification of allergen-specific IgE in serum by the FEIA and CAP methods (Pharmacia Diagnostics AB, Uppsala, Sweden) [5].

The nonanaphylactic deaths (n=55) comprised a control group of sudden cardiac fatalities, deaths by acute aortic dissection (aad), and another group of deaths caused by asphyxia (compression of the chest, positional asphyxia, and smothering) (Table 1).

Tryptase was measured with a commercial tryptase fluoroenzymeimmunoassay (FEIA) method (Pharmacia Diagnostics AB, Uppsala, Sweden).

Assuming that tryptase concentrations in femoral blood in the group of sudden cardiac deaths were gamma distributed [13]; the distribution was estimated with the maximum likelihood method and an upper cut off value, covering 95% was calculated.

A maximum-likelihood technique was used for 5,000 random samples from the data set. Also the standard deviation (SD) for the cut-off value was calculated by this method. The MATLAB program (The MathWorks, Massachusetts, USA) was used for the computations. The Statview program (Abacus Concepts, Berkeley, CA) was used for statistical analyses, i.e., paired t test, simple linear regression, the Mann–Whitney U test, and Fisher's exact test for nonparametric data.

Results

Individual data concerning tryptase concentrations in the diagnostic groups are seen in Table 1 and Fig. 1. The tryptase values in serum from femoral and heart blood varied greatly with statistically significant difference in the whole material (p<0.026) and in the control group (p<0.023) but not in the asphyxic or anaphylactic deaths (p>0.05). Simple linear regression showed a strong positive correlation only in the anaphylactic cases, r=0.78.

Using the tryptase concentrations in femoral blood from the control group (sudden cardiac deaths) as the standard regarding the data as a sample from a gamma distribution, 95% of the distribution was estimated to be lower than 44.3 μg/l. This was then used as the cut-off value in the nonparametric tests. The SD for the cut-off value was 5.3 (Fig. 2). For heart blood the corresponding values were 93.2 μg/l and SD 18.0.

A significant difference was seen between anaphylactic deaths and the control group regarding tryptase concentrations in femoral blood (p<0.007) but not in heart blood (p>0.05)(Table 2).

In the asphyctic deaths, the mean tryptase concentration in femoral blood was 28.4 μg/l which was significantly different from that in the controls (p<0.038) (Table 2).

No statistically significant influence was seen on tryptase concentrations by prone position, petechiae in the face and conjunctivae, or resuscitation efforts by means of cardiac massage either in femoral or heart blood as measured with the Mann–Whitney U test or Fisher's test.

Discussion

High serum tryptase concentrations have previously been noted in quite a few individuals who died from nonallergic causes [6, 7]. This is especially interesting in victims of sudden infant death syndrome (SIDS) [10–12], acute deaths after injection of heroin [14, 15], and traumatic deaths [9], where 30–40% of cases show high tryptase values. We have previously reported that SIDS infants who died in a prone position tended to have higher tryptase values than those found in a supine position [12]. In the present study, prone position had no influence on the tryptase concentrations in blood in adult subjects. On the other hand, blood from SIDS victims is regularly sampled from the heart chambers due to the scarcity of blood in the femoral veins. Our results showed that samples from heart blood had significantly higher tryptase concentrations compared with femoral blood. This fact offers one explanation for the increased values that have been observed in SIDS. The increased

Table 1 Details of the 16 deaths in asphyxia, 39 cases of sudden cardiac deaths and acute aortic dissection (controls), and five anaphylactic deaths

	Tryp fem ug/l	Tryp cor ug/l	Diagnosis	Petechiae	Position	Resuscitated	Gender	Age
1	12		Asphyxia	No	Prone	No	M	65
2	31		Asphyxia	Yes	No	Yes	M	35
3	31	23.5	Asphyxia	Yes	No	No	M	46
4	19	56	Asphyxia	Yes	No	No	M	44
5	10	33	Asphyxia	Yes	Prone	No	F	40
6	32	48	Asphyxia	Yes	No	No	M	57
7	25		Asphyxia	No	Prone	No	F	61
8	29	17	Asphyxia	Yes	No	No	M	28
9	26	35	Asphyxia	Yes	No	Yes	M	63
10	57	56	Asphyxia	Yes	Prone	No	M	49
11	20	21	Asphyxia	No	Prone	No	M	65
12	47	180	Asphyxia	Yes	Prone	No	M	23
13	57	20	Asphyxia	No	Prone	No	M	44
14	7.7	11	Asphyxia	No	Prone	No	F	66
15	38	15	Asphyxia	No	Prone	No	M	39
16	13	55	Asphyxia	No	Prone	No	F	58
17	15	24	scd	Yes	Prone	No	F	22
18	31	150	scd	No	Prone	No	M	54
19	6	11	scd	No	Prone	No	F	38
20	28	32	scd	Yes	No	No	M	57
21	23	48	scd	Yes	Prone	No	M	55
22	24	160	scd	Yes	No	No	M	33
23	5.6	6.6	scd	No	Prone	No	M	52
24	14	21	scd	No	Prone	No	M	70
25	33	30	scd	Yes	Prone	No	M	82
26	19	7.8	scd	Yes	Prone	No	M	24
27	9	20	scd	No	No	Yes	M	51
28	23	47	scd	No	No	Yes	M	48
29	55	48	scd	No	No	No	M	20
30	9	26	scd	No	No	Yes	M	14
31	13	16	scd	Yes	No	Yes	M	18
32	21	17	scd	No	Prone	No	M	37
33	7	7	scd	No	No	No	F	40
34	8		scd	No	No	No	M	47
35	15		scd	No	No	Yes	F	47
36	11	55	scd	No	No	Yes	M	53
37	41		scd	No	No	Yes	M	8
38	9	10	scd	No	No	No	M	41
39	40	25	scd	No	No	Yes	F	27
40	26		scd	Yes	No	No	M	65
41	11		scd	No	No	Yes	M	26
42	12	11	scd	Yes	No	No	M	44
43	3	2	scd	No	No	No	F	59
44	16		scd	No	No	Yes	M	48
45	41		scd	No	No	No	F	39
46	39		scd	No	No	Yes	M	49
47	17		scd	No	No	Yes	M	12
48	18	5	scd	Yes	No	No	M	84
49	19	13	scd	No	No	Yes	M	20
50	23	14	scd	No	No	Yes	M	15
51	10	30	sd-aad	No	No	Yes	F	47
52	6		sd-aad	No	No	No	M	56
53	45		sd-aad	No	No	Yes	M	47

Table 1 (continued)

	Tryp fem ug/l	Tryp cor ug/l	Diagnosis	Petechiae	Position	Resuscitated	Gender	Age
54	3		sd-aad	No	No	Yes	M	51
55	19	59	sd-aad	No	No	Yes	M	54
1	13	18	Anaphylaxis	No	No	Yes	M	81
2	72	37	Anaphylaxis	No	No	Yes	M	15
3	200	170	Anaphylaxis	No	No	No	M	47
4	320		Anaphylaxis	No	Prone	No	M	55
5	180	390	Anaphylaxis	No	No	Yes	M	50

Tryp fem Tryptase concentration in blood from femoral vessels; *tryp cor* tryptase concentrations in blood from the heart chambers; in the column diagnosis, *asphyxia* includes positional asphyxia, smothering, and external compression of the chest, *scd* sudden cardiac death, and *sd-aad* sudden death by acute aortic dissection

tryptase concentrations seen in heroin deaths and trauma [9, 14, 15] cannot be ascribed to sampling site.

Our finding that death by asphyxia caused significantly elevated tryptase concentrations in femoral blood is consistent with previous impressions. Laboratory studies have also shown that systemic hypoxia activates the mast cells in the rat [16]. The phenomena generally associated with asphyxia before death (e.g., conjunctival petechial bleeding, prone position) were not associated with increased tryptase and as they are found in various circumstances, these factors may be regarded as nonspecific. Also, postmortem events such as mechanical compression of the chest (resuscitation) influenced neither the heart nor the femoral blood concentration of tryptase.

We might recognize only a few of all the factors that must be accounted for when evaluating individual measurements of tryptase (Table 3). The significantly higher tryptase concentration in heart blood compared with femoral blood in the controls could be explained by a passive diffusion of tryptase from the numerous mast cells in the lungs and heart that might occur postmortem. The fact that this difference was not observed in anaphylactic deaths, where a strong positive correlation was seen between heart blood and femoral blood, is theoretically consistent with the knowledge that tryptase release by mast cell degranulation plays a dominant role in anaphylaxis. Because we do not yet know all the factors affecting the mast cells postmortem, the lack of correlation between heart and femoral blood in the control group remains to be explained. It is obvious, however, that that sampling of blood from the heart chambers for tryptase analysis should be avoided. One of the five anaphylactic deaths presented in this study showed normal tryptase concentrations in both samples. In this case, the circumstances of death and the finding of allergen-

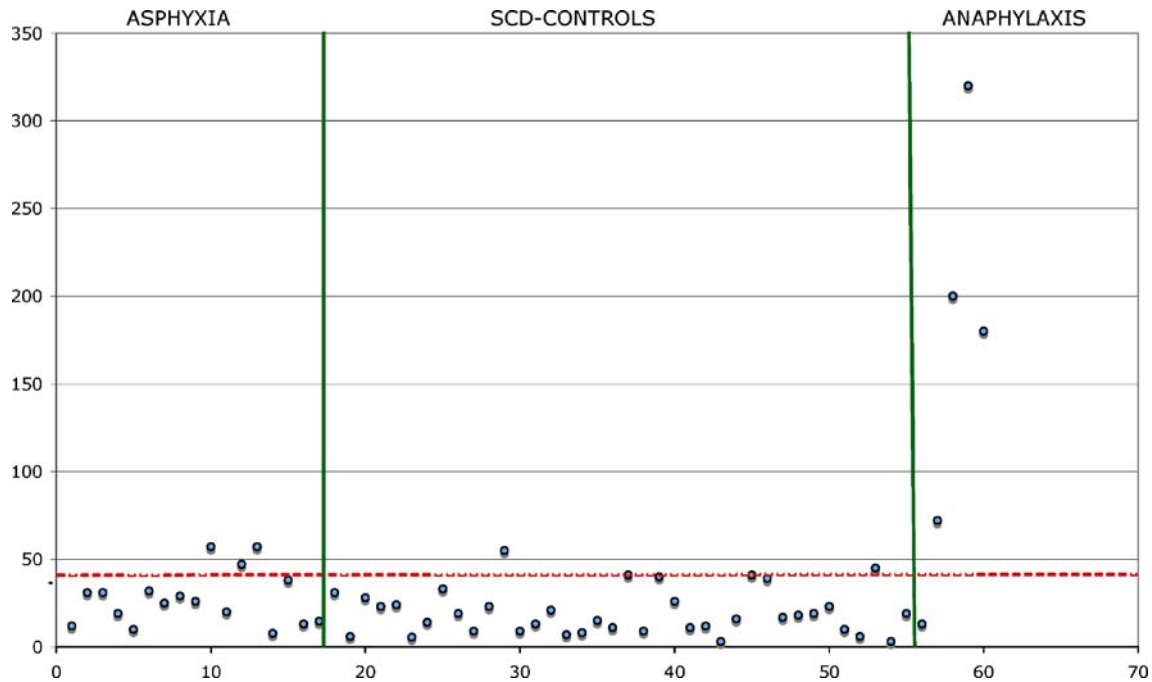

Fig. 1 Point diagram showing individual tryptase values in asphyxic deaths, controls (sudden cardiac death and acute aortic dissection), and anaphylactic deaths on the *x*-axis and tryptase concentration μg/l on the *y*-axis. The *horizontal broken line* denotes the upper normal limit 44.3 μg/l

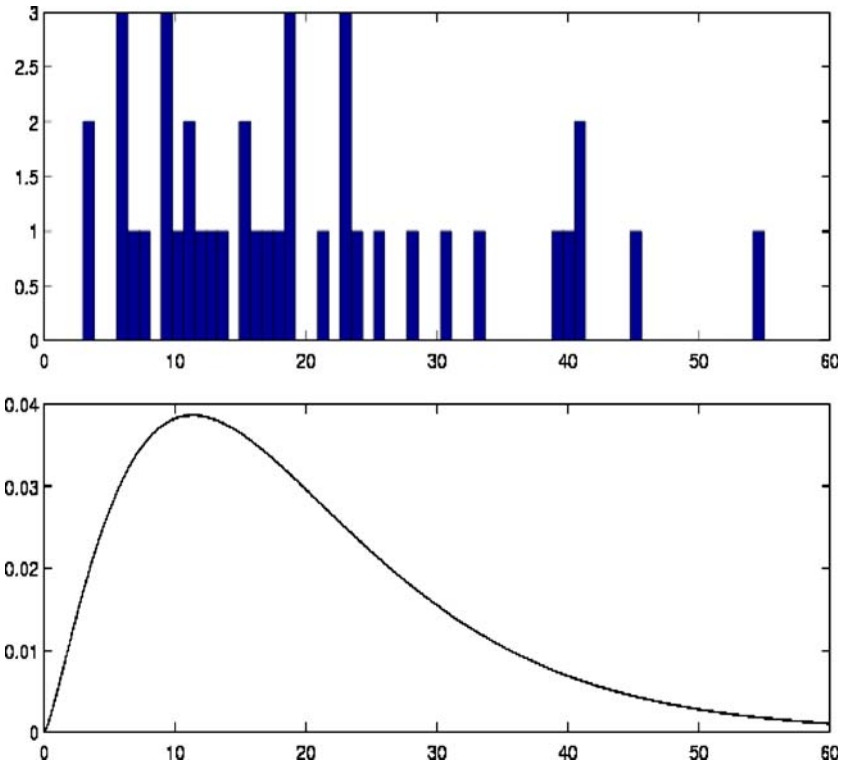

Fig. 2 Individual tryptase values in femoral blood in 39 cases (**a**) fitting a gamma distribution curve (**b**). Normal values (95%) are below 44.3 μg/l. *X*-axis shows tryptase concentration in serum μg/l and *y*-axis the number of cases within each integer

specific IgE corresponding to known exposure to the allergen must have precedence over tryptase concentrations per se. From previous studies, it is also known that in food anaphylaxis, for example, tryptase is not or only moderately elevated [4].

The wide range of possible confounding factors complicates the selection of a proper control group. In this study, the cases were not selected randomly as we wanted to study the effect of certain variables that we suspected of influencing the tryptase concentrations postmortem. Compared with clinical reference values that can be established by analysis of numerous healthy individuals under standardized conditions, each postmortem subject is unique regarding the circumstances around death and the postmortem environment until autopsy. However, previous experience has shown that elevated tryptase values in femoral blood are rare in sudden cardiovascular fatalities [6], and in rapid deaths occurring within minutes of the assault even if mast cells were degranulated, at least in theory, tryptase would not have had time to be released into the circulation [17].

With the statistical method used in this study, we defined an upper normal limit for tryptase in femoral blood which is recommended for interpretation of postmortem concentrations. The upper reference value was found to be rather high, which might increase the probability of false negatives, one case (20%) in our small sample of five anaphylactic deaths. The distribution of tryptase values (Fig. 2) was found to have a close fit with a gamma-distribution curve [13]. The values were treated as a random sample, and an estimate of an upper cut-off limit was calculated. The standard error of the cut-off value was estimated by simu-

Table 2 Tryptase μg/l (SD) in serum from blood samples taken from the femoral vessels and the heart chambers

	n	Tryptase femoral blood (SD)	*n*	Tryptase heart blood (SD)
Controls	39	19.7 (12.8)	27	33.2 (38.6)*
Asphyxic deaths	16	28.4 (15.4)**	13	43.9 (44.1)
Anaphylactic deaths	5	157.0 (119.3)***	4	153.8 (171.4)

*Statistically different from femoral blood control group (paired *t* test $p<0.043$)
**Significantly different from femoral blood control group ($p<0.038$, Mann–Whitney *U* test)
***Significantly different from femoral blood control group ($p<0.007$, Mann–Whitney *U* test)

Table 3 Some factors influencing tryptase blood concentrations in vivo and postmortem

Influences on mast cell activation, degranulation, and tryptase release in vivo [19], and postmortem confounders
Anaphylaxis
Bacterial infections
Opiates
Contrast media
Analgesics
Mastocytosis/urticaria pigmentosa
Exercise/stress
Postmortem time [8]
Hemolysis/autolysis [9]
Trauma [9]
Sampling site (*present study*)
Premortem asphyxia (*present study*)

Possible confounders in italics

lation [18]. The precision of those values could be increased further by inclusion of additional control cases. This statistical method could also be used to establish reference values for postmortem measurements of other substances with other types of distributions. But it is important to carefully choose a distribution that will fit the data as closely as possible.

Mast cell tryptase is primarily thought of as a marker for systemic IgE-mediated hypersensitivity reactions. In this and previous studies, we have shown that (1) tryptase is generally higher in postmortem samples than in serum from living patients, (2) in evaluating individual tryptase concentrations, many confounding factors must be accounted for, (3) significant mast cell release of tryptase from nonIgE-mediated stimuli occurs that might be a cause of or contribute to death, and (4) if the upper normal reference limit is set rather high, <45 μg/l, it will eliminate most false positives. Statistically, a larger population of certain anaphylactic deaths is needed to safely assess the fraction of false negatives at that reference limit.

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